

**In the Claims:**

Above claim 1, insert the following:

What is claimed is:

**REMARKS**

The foregoing amendments are presented to place the application in compliance with the sequence rules under 37 CFR 1.821-1.825.

Applicants have submitted a Sequence Listing in both paper and computer readable form as required by 37 C.F.R. 1.821(c) and (e). Amendments directing its entry into the specification have also been incorporated herein. The content of the paper and computer readable copies are the same and no new matter has been added.

The specification has also been carefully reviewed and editorial changes have been effected. All of the changes are minor in nature and therefore do not require extensive discussion. Specifically, the specification headings have been amended in conformance with U.S. practice.

Also, the amino acid sequences disclosed in Figure 4 which represent portions of SEQ ID Nos: 9-13 have been identified and labeled in the Brief Description of the Drawings (See Appendix A) in accordance with U. S. practice.

With regard to the Notice also requesting that an executed Oath and Declaration be submitted, Applicants wish to note that an executed Oath and Declaration was submitted on May 29, 2002. A copy of the submitted executed Declaration is enclosed herewith along with the cover letter (indicating the filing of the executed Declaration). Applicants respectfully request that the

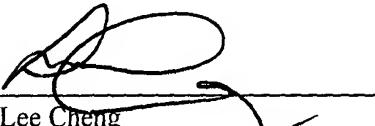
Patent Office review the application papers to ensure that the executed Declaration is present in the file.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "Version with markings to show changes made."

In view of the foregoing, it is believed that each requirement set forth in the Notice has been satisfied, and that the application is now in compliance with the sequence rules under 37 CFR 1.821-1.825. Accordingly, favorable examination on the merits is respectfully requested.

Respectfully submitted,

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## APPENDIX A

The amino acid sequence of HP01347 shown in Figure 4 corresponds to amino acid residues 1-72 of SEQ ID No: 9. The amino acid sequence of HP10328 shown in Figure 4 corresponds to amino acid residues 1-128 of SEQ ID No: 10. The amino acid sequence of HP10390 shown in Figure 4 corresponds to amino acid residues 1-50 of SEQ ID No:11. The amino acid sequence of HP10433 shown in Figure 4 corresponds to amino acid residues 1-135 of SEQ ID No: 12. The amino acid sequence of HP10481 shown in Figure 4 corresponds to amino acid residues 1-148 of SEQ ID No: 13.



DESCRIPTION

**A Method for producing an Antibody by Gene Immunization**

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BACKGROUND OF THE INVENTION

1. Technical Field of the Invention

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The invention of the present application relates to a method for producing an antibody by gene immunization. More specifically, the invention relates to a method of enabling easy production of an antibody useful as drugs, diagnostic agents, reagents for the research, and etc., and to an expression vector used in this method.

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part 2  
A3  
Description of the Related  
Background Art

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An antibody has widely been utilized as reagents for the research for the purpose of detection, purification, elimination, inhibition of a protein or the like, because it has property of recognizing specific protein and binding thereto. Recently, it has widely been used not only as reagents for the research but also as drugs or diagnostic agents.

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In producing antibodies, it has so far been general to use a method that a large amount of protein as an antigen is purified and injected to an animal or animals such as rabbits or mice to collect antibodies generated in sera. It required, however, much time and a great deal of labor to obtain a large amount of a purified antigenic protein. It is desired to provide a more convenient method for producing antibodies, accordingly.

Recently, it was reported that when a gene coding for an influenza virus nucleoprotein is integrated into an expression vector

and intramuscularly injected directly as DNA to mice, then virus proteins are produced in the murine bodies and additionally the antibody against these proteins are generated in the sera. (Ulmer et al., Science 259: 1745-1749, 1993; Ginsbert et al., "Vaccines 93"). As a result, this expression vector received much attention as a new type of vaccine, that is, DNA vaccine, since mice have acquired immunity to virus. Thus, it has been designated as gene immunization that an expression vector for an antigenic protein is inoculated directly to an animal to generate immunity. In using gene immunization, however, in some cases, the titer of the generated antibody is very low or no antibody is generated depending on the kind of the antigen used.

It was reported as an example of gene immunization that ovalbumin was fused in the downstream of transmembrane domain of transferrin receptor to form a membrane type and it was injected intramuscularly or subcutaneously to mice in order to investigate an effect of the expression site of antigenic protein on the efficacy of gene immunization. The titer of the antibodies generated, however, rather decreased since the protein was converted into a membrane type. (Boyle et al., Int. Immunol. 9: 1897-1906, 1997).

*As of*  
The purpose of the invention of present application is to provide a method for producing antibodies to proteins, which <sup>it was</sup> ~~were~~ difficult to produce <sup>using</sup> ~~presently~~ in-so-far known gene immunization methods.

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Additionally, the purpose of the invention is to provide an expression vector used in the above-mentioned method for producing an antibody.

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*AB* **Summary  
Disclosure of the Invention**

The present application, as the invention for solving the

Fig. 4 shows the respective N-terminal amino acid sequences of fusion proteins comprising urokinase and transmembrane domains in a variety of membrane proteins.

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*- Insert "Appendix A"*

~~Diagram~~  
*Description of the Preferred Embodiments*  
*Best Mode for Carrying Out the Invention*

In a method for producing antibodies according to the invention, 10 the expression vector to be inoculated to animals may be constructed as an expression vector having a fusion polynucleotide that consists of a polynucleotide encoding an antigenic protein and a polynucleotide encoding a transmembrane domain.

15 As for an antigenic protein, any one that can generate an antigen-antibody reaction in vivo may be used. The polynucleotide encoding an antigenic protein may be any one of genomic DNA, cDNA, synthetic DNA, etc., as far as it has an open reading frame (ORF). When the antigenic protein is an inherent secretory protein, it is used 20 after removal of the signal sequence peptide originally possessed by the protein.

As for the transmembrane domain, any domain may be used as far as its N-terminal side is in the cell and the C-terminal side is out of 25 the cell. For example, transmembrane domains of type II-membrane proteins or those of multispan-type membrane proteins may be used. The proteins that an antigenic protein is fused to the C-terminal side of these transmembrane domains take forms that the antigenic protein portion exists on the surface of the cell membrane. As for the 30 transmembrane domain, for example, that of human type-II membrane protein HP10085 (SEQ ID NO: 2) may be used. In this case, the transmembrane domain to be fused with an antigenic protein is a polypeptide containing at least 1st methionine (Met) to 26th lysine (Lys)

immunoassay (ELISA), Western blotting, immuno-precipitation, antibody staining, and the like may be used. After confirmation of the presence of the antibody in the serum by these methods, the serum may be used as a polyclonal antibody specimen as it is or may be purified by 5 affinity column chromatography to yield IgG. Alternatively, the spleen may be taken out from the animal acquiring immunity and the monoclonal antibody can be produced in a conventional manner.

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### Examples

~~The following examples serve to illustrate the invention in more detail and specifically but are not intended as a limitation thereof. In these examples, basic procedures for recombination of DNA and enzyme reactions are carried out according to the articles, "Molecular Cloning; A laboratory manual", Cold Spring Harbor Laboratory, 1989. Restriction enzymes and a variety of modified enzymes were obtained from Takara Shuzo Co., Ltd., unless otherwise stated. The compositions of buffer solutions in respective enzyme reactions and the reaction conditions were set according to the specification attached.~~

25 (1) Construction of an Expression Vector for the Urokinase-Fusion Protein

When urokinase is used as an antigenic protein, 3 kinds of expression vectors were used, that is, for secretion expression, for membrane form expression, and for intracellular expression. That is, the following vectors were respectively used: for secretion expression, 30 pSSD1-UPA22 which expresses the signal sequence and protease domain of urokinase (Yokoyama-Kobayashi et al., Gene 163: 193-196, 1995); for membrane form expression, pSSD3-10085H which expresses a protein prepared by fusing a sequence from the N-terminal side to the

Industrial Applicability

~~According to the present invention, an antibody against an antigenic protein, which it was difficult to produce in the so far known gene immunization, can be produced. The resulting antibody is useful as drugs, diagnostic agents, and reagents for the research.~~

## CLAIMS

What is claimed is,

1. A method for producing an antibody which comprises inoculating an expression vector expressing a fusion protein to an animal, isolating an antibody against an antigenic protein from the animal and purifying the antibody, wherein the fusion protein is an antigenic protein fused with the C-terminal of a transmembrane domain of which the N-terminal side is located in the cell and the C-terminal side is out of the cell.
- 10 2. The method for producing an antibody of claim 1, wherein the transmembrane domain is a polypeptide having at least the amino acid sequence from 1st to 26th of SEQ ID NO. 2.
- 15 3. An expression vector expressing a fusion protein in which an antigenic protein is fused with the C-terminal of transmembrane domain of which the N-terminal side is located in the cell and the C-terminal side is out of the cell.
- 20 4. The expression vector of claim 3, wherein the transmembrane domain is a polypeptide having at least the amino acid sequence from 1st to 26th of SEQ ID NO. 2.

*part A 9*  
ABSTRACT OF THE DISCLOSURE

*part A 10* The present invention of the application provides a method for producing an antibody which comprises inoculating an expression vector expressing a fusion protein to an animal, and isolating and purifying an antibody against an antigenic protein from the animal, wherein the fusion protein is an antigenic protein fused to the C-terminal side of a transmembrane domain in which the N-terminal side is located in the cell and the C-terminal is out of the cell.

10 According to the present invention, an antibody against an antigenic protein, which ~~it~~ was difficult to produce <sup>using present</sup> in the so far known gene immunization, can be produced. The resulting <sup>is</sup> antibody is useful as drugs, diagnostic agents, and reagents for the research.